A STUDY OF THE CONTENT AND MICROBIOLOGICAL EFFECT OF SOME SPROUTS AND THEIR DIETARY IMPORTANCE

Doctoral (PhD) thesis

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INTRODUCTION

Consuming vegetables and fruits mostly raw provides not only dietary minerals, vitamins and fibre, but also bioactive compounds beneficial for the human organism. During the summer and autumn months a wide variety of vegetables and fruits grew on open air allows for sufficient vitamins and dietary minerals. During the winter and early spring months we only have produce and foodstuff grew in glasshouses or import harvested unripe and transported over long distances. Their vitamin content, chemical composition and flavour differs from local, openly grew plants, and because of their higher price less people consume them. The consumption of germinated plants, which can be prepared in little time (approximately one week), is widespread in many countries. It is consumed raw in salads and sandwiches. They are available at comparatively low prices, or can be grown at home, however in Hungary the tradition of consuming sprouts is lacking. Bioactive compounds are present in growing plants at a higher concentration. These phytochemicals can be beneficial not just for the plant, but by consuming the germinating plant for the human organism as well. Bioactive compounds that are able to prevent development of cardiovascular diseases and some tumours, and have antimicrobial attributes have been detected in vegetables and fruits. Flavonoids, glycosynolades and lignans are among the bioactive compounds. Nowadays we can get information on the bioactive components different vegetables and fruits from an electronic database (for the EuroFIR-European Food Information Resources this is the eBASIS-BioActive Substance in Food Information System). This system, however, does not contain the complete data on sprouts yet.

The consumption of raw vegetables and fruits, according to epidemiological research data, significantly lowers the risk of carcinogenesis.

Despite the proper diet, it is not rare that some infection develops in people; either enteric when infectious agents enter with food or through the respiratory tract, or with surgical and medical procedures. Broad spectrum antibiotics with different modes of action are used more and more against bacterial infections. The widespread use of antibiotics entails several risks, as they destroy the beneficial bacteria as well as those harmful to the organism, and have several side effects. The biggest problem however is the selection of multidrug resistant microorganisms. Despite developing newer and newer generations of medicine, their use selects for the resistant bacteria, which multiply, pass their resistant genes over, often making the therapy ineffective. It is important to prevent infections, or else that the treatment of developed infections does not cause the selection for multidrug resistant microorganisms, or

lead to another infection (for example diarrhoea caused by Clostridium difficile). A possible method of prevention is having a proper diet and lifestyle.

AIM

The aim of our research was to analyse the effects of sprout consumption that is in the fore of modern diet. The nutritional values of the sprouts during the growth period can change by using up the proteins, fat and carbohydrates. Thus we made it our aim to determine how the nutritional values change during the growth, and short storage of sprouts.

In the course of our work we wanted to investigate the flavonoid content of the sprouts, as the literature mentioned in the introduction confirm that flavonoids have a beneficial effect on the human organism. By comparing the methods used to detect flavonoid content we determined which method is the best for investigating the flavonoid content of sprouts. Assessing the flavonoid compounds in plant germs can help us decide which sprout is the most effective, which can be used and suggested in a preventive diet. Some vegetables contain antimicrobial compounds. We wanted to test which plants' germs have these attributes by employing microbiological methods, so that the sprouts can possibly help destroy the causative agents of enteric bacterial infections.

Furthermore, we aimed to clarify whether the sprouts' compounds can prevent the development of infections in case of a bacterial contamination.

With our study we wanted verification that the consumption of the sprouts of some plants is more beneficial in health promotion. There has been no survey of the consumption of dietary sprouts in our nation, and since we are convinced, that the beneficial effects of sprout consumption is not well known enough, it was our aim to examine the range and consumption possibilities of dietary sprouts in Hungary.

RESEARCH MATERIAL AND METHOD

We germinated 55 different untreated vegetable and herb seeds available in the marketplace. The seeds originated from the Rédei Kertimag Vetőmagkereskedelmi Zrt. (Réde, Hungary), the Zöldségtermesztési Kutató Intézet Zrt (Kecskemét, Hungary) and the Szentesi Mag Kft. (Szentes, Hungary). The bacteria strains used in the study were from the strain collection of the Department of Immunology and Biotechnology of the University of Pécs Medical School.

The plant sprouts were examined according to the following methods:

1. Examination of the effects of homogenized plant sprouts

Agar diffusion method for screening the antimicrobial effects of the sprouts

Examination of the pathogenic germ survival in homogenized radish sprouts' tenfold dilution

2. Determination of the nutritional value of the seeds and sprouts with chemical methods

Determination of the water content of the seeds with gravimetric methods

Determination of the water content of the sprouts with gravimetric methods

Determination of the fat content with Soxhlet extraction

Determination of the protein content with Kjeldahl-method

Determination of the full carbohydrate content (after hydrochloric acid hydrolysis and Schoorl's iodometric method)

Determination of the iron content (with tiocianate method, spectrophotometer)

Determination of the potassium content

Determination of the sodium content

Determination of the calcium content

Determination of the vitamin C content with α, α'-dipiridil method

3. Determination of the full-flavonoid content according to the VIII. Magyar Gyógyszerkönyv (Ph. Hg. VIII.):

Determination of the O-glycosides (according to the Solidaginis herba article of the Ph. Hg. VIII.)

Determination of the C-glycosides (according to the Crataegi fructus article of the Ph. Hg. VIII.)

4. Thin-layer chromatographic examination of the flavonoids
5. Questionnaire survey to examine how prevalent are sprouts and how they are consumed.

RESULTS

Examination of the antibacterial effects of plant sprouts

55 different seeds were germinated for a week, then the parts above the radicle were collected and masticated with blender homogenizers, and it was determined with agar diffusion method which plant sprouts block, and to what extent, the bacteria strains used in the study. Ten plants sprouts, which had shown an antibacterial effect on several bacteria, were selected. Radish sprouts were the most effective. Munich beer radish and black radish sprouts blocked the growth of bacteria exceptionally well. Of the members of the *Brassicaea* family kohlrabi and red cabbage were similarly effective in blocking the growth of the examined bacteria. The sprouts of the mustard plant, zucchini, camomile, fenugreek, adzuki bean had antibacterial effect on the *Staphylococcus aureus* strains and on some Gram-negative intestinal bacteria, but had no effect on the examined human enteric bacteria. (Table 1)

Table 1. The effect of sprouts on the reproduction of bacteria

	Kohlrabi	**Red cabbage**	**Mustard plant**	**Easter rose radish**	**Black radish**
ETEC	++	+	-	+	++
EIEC	++	+	-	+	++
EHEC	+	+	+	+	++
***S.* Typhimurium**	++	+	+	+	++
S. flexneri	++	+	-	+	++
***E. coli* ATCC 25922**	++	+	-	+	++
P. mirabilis	++	+	-	+	++
K. pneumoniae	+	+	-	+	+
Enterobacter sp	++	+	-	++	++
***S. aureus* ATCC 23923**	++	+	+	+	++
MRSA	++	+	+	+	++
***P. aeruginosa* ATCC 27387**	-	-	-	-	-
	Icicle radish	**Munich beer radish**	**Fenugreek**	**Scorzonera**	**Zucchini**
ETEC	**+**	**++**	**-**	**-**	**-**

EIEC	+	++	+	-	-
EHEC	-	++	-	-	-
***S.* Typhimurium**	+	++	-	+	-
S. flexneri	+	++	-	-	-
***E. coli* ATCC 25922**	-	++	++	-	-
P. mirabilis	+	++	-	-	-
K. pneumoniae	+	++	-	-	-
Enterobacter sp	+	++	+	+	-
***S. aureus* ATCC 23923**	-	++	++	-	+
MRSA	-	++	+	-	+
***P. aeruginosa* ATCC 27387**	-	-	-	-	-

+: Diameter of the zone of inhibition: 9-12 mm ++: Diameter of the zone of inhibition: 12-22 mm -: Zone of inhibition did not develop

Twenty-nine herb and plant sprouts had no inhibitory effect on the growth of bacteria. Whether the radish sprout, after having been contaminated with a small number of enterohemorrhagic Escherichia coli strains and homogenized or diluted in medium, has an antibacterial effect and the bacteria die, or the bacteria are still present, was tested. The antibacterial effect of the radish sprout did not prevail, the EHEC strains reproduced in the tenfold medium dilution of the sprout.

Changes in the nutritional values of the sprouts

The plant uses the nutrients stored in the seed to develop the sprout, until it can produce for itself through photosynthesis. As a point of departure the nutritional values of the seeds were determined, and used to compare the nutritional values of three day and one week old sprouts. The nutritional value changes in developed, one week old sprouts, fit for consumption, were determined after three days' storage. The protein, fat and carbohydrate content decreased in radish sprouts after growth and storage, but the ascorbic acid content increased. Among the examined seeds and sprouts the radish sprout had the highest vitamin C content. Vitamin C was present in the sprouts in much smaller quantities (35.46 mg and 33.08 mg/100 gram) than in the seeds (94.03 mg/100 gram). The mineral content of radish sprouts was similar to alfalfa sprouts (Na=102 mg, Ca= 122 mg, K= 106 mg, Fe= 1, 25 mg). Wheat sprouts had a lower protein (3.3 g), fat (0.26 g), carbohydrate (11.56 g) and vitamin C (10.42 mg) content

compared to the seed (15.6 g). During the growth period the fat content remained unchanged (0.26 g), the vitamin C content increased (10.32 mg). Of the minerals the sodium content remained unchanged (42 mg). Mung bean sprouts also had a lower protein (5.26 g), fat (1.00 g), carbohydrate (9.08 g) and vitamin C (12.94 mg) content than the seed. During the growth period these nutritional values decreased even further. The three day storage period did not affect the nutritional value significantly. Compared to the seeds, the sprouts' mineral content decreased. During the growth period the sprout's potassium (106 mg) and calcium (5.00 mg) content decreased slightly, and the iron (0.66 mg) and sodium (46 mg) content narrowly increased. Fenugreek's sprout, similarly to other sprouts, had lower protein, fat, carbohydrate and vitamin C (6.64 mg) content, than its seed. During storage the values did not change significantly, except from the full vitamin C content, which did decrease significantly (2.1 mg). The mineral content in the sprouts is significantly lower. The potassium content (67 mg) decreased even more during germination (121 mg) and storage (79 mg), while the other mineral content values did not change significantly.

Determination of the full flavonoid content

The spectrophotometric method, capable to detect O-glycosides, showed low values of full flavonoid content in radish, wheat, broccoli and alfalfa, and showed no flavonoid content in buckwheat, sunflower, mung bean and rye sprouts. The method capable to detect C-glycosides also showed very low full flavonoid content in mung bean and onion sprouts and no flavonoid content in sunflower sprouts. Only the broccoli sprouts had more C-glycosides, but even these values were low (0.12%). The flavonoids determined with the two spectrophotometric methods were not detected in significant quantity in either of the sprouts. The full flavonoid content of the sprouts differed. In case of the sunflower neither method detected any flavonoid content. By comparing the two methods we can establish that in the case of the broccoli and mung bean sprouts the C-glycoside examination was more efficient, as the broccoli showed four times as many C-glycosides as O-glycosides, and the mung bean sprouts only showed C-glycosides. Of the examined sprouts, the flavonoid content of the broccoli is the highest (albeit still in small percentages), as both O- and C-glycosides were detected. Four phenolic compounds, belonging to different categories, were identified with thin layer chromatographic examination of the flavonoids of the sprouts, when compared with standard compounds. The chlorogenic acid, belonging to the phenol carbolic acids, was detected in three sprouts, while caffeic acid only in the sunflower sprouts. Of the O-glycoside flavonoids, rutin was detected in buckwheat sprouts, but hyperoside was not detected in any

of the sprouts. Of the C-glycoside flavonoids orientin and vitexin showed only in the buckwheat sprout samples. Luteolin and apigenin, belonging to the flavone class, was not detected in any of the sprouts.

This layer chromatographic examination of the flavonoids

We managed to examine eight sprouts with thin layer chromatography. According to the thin layer chromatography the sprouts contain different flavonoid compounds and in different quantities. The flavonoids appearing on the chromatograph were only identified in the case of the buckwheat, namely rutin and orientin, and a vitexin spot was barely visible. Hyperoside, luteolin and apigenin was undetected in the sprouts. In case of the wheat, onion and alfalfa sprouts no visible spots were on the layer, which suggests, that with thin layer chromatography the flavonoids in them are unidentifiable. Therefore, based on the colour and the R_f value of the standard compounds, it is probable that sunflower sprouts contain caffeic acid and chlorogenic acid, broccoli and radish sprouts contain chlorogenic acid, and buckwheat sprouts contain rutin, vitexin and orientin.

Questionnaire survey

250 questionnaires were filled in after random, stratified sampling, of which 200 was utilizable. 44% of the sample was male, 56% female. The lower age limit was 18, the higher 46 for men and 44 for women. The beneficial effects sprouts exercise on the body is plentiful. 92 % of the sample knows some of the beneficial effects of the sprouts. Most well-known is the high vitamin and mineral content, of which 24% of the sample was familiar with. There is a wide range of seeds for germination and germinated plants on the market. Despite the large variety the range of consumed sprouts is slim. According to the answers of regular consumers, the wheat sprout is the best known. In addition, the consumption of radish (33%), alfalfa (22%), corn (17%) and onion (14%) sprouts is prevalent. Despite their valuable nutritional content other sprouts are not consumed. Regarding the frequency of sprout consumption there is no significant variation among the answers. Our hypothesis, that the flavour is the decisive factor when choosing which sprout to consume, was confirmed by the regular sprout consumers. At a lower degree, but their decision is also influenced by the physiological effects of the different sprouts, and the nature of the food cooked from them. The outlook of the different sprouts had the least import in their answers.

DISCUSSION, CONCLUSION

It was confirmed through examination, that some of the plant sprouts, especially homogenized sprouts of the different varieties of radish, kohlrabi and red cabbage exercise an antibacterial effect on most of the bacteria used in the research. These belong to the same family plants, of which it was proven for full-grown plants that in case of tissue injury sulphurous sulforaphane and thyocianate compounds are released by the myrosinase enzyme. The homogenization injured the plants, thus activating the enzyme and creating the antibacterial compounds. However, the quality and quantity of the antibacterial compounds may vary, as they had various effects on the different strains of bacteria. The antibacterial agents incipient on the homogenization of the sprouts had no effect on *Pseudomonas aeruginosa*, but they had an antimicrobial effect on human enteric pathogenic bacteria strains. Radish sprouts had the most significant antibacterial effects; however differences were shown in the antimicrobial effectiveness of the various radish sprouts. The sprout of the daikon radish inhibited the spread of the enterohemorrhagic *Escherichia coli*, MRSA and the strains of *S aureus*. As the myrosinase enzyme is only released on tissue injury and the antibacterial isothiocyanate is exuded by it, it could not have an effect on the bacteria. The tenfold dilution of the homogenized radish sprout, which had an antibacterial effect on the EHEC strain, in medium, was contaminated with a low germ count EHEC strain. The bacteria accrued, the antibacterial effect did not prevail. On mastication the tissues of the radish sprout, the exuded antibacterial compounds might destroy low germ count bacteria, but when consumed with other food or drink the effect does not prevail. For similar reasons the antibacterial compounds presents in the sprouts have no effect in the stomach. 52% of the examined sprouts had no antibacterial effects on the examined bacteria, therefore, when contaminated they may cause food infection, food poisoning. Mustard, zucchini, camomile, fenugreek and adzuki bean sprouts had no antibacterial effect on the enteric pathogen strains, but they had an effect on the *S aureus* strains. Hygiene cannot be neglected during germination either at home, or when it is produced in bulk. Decontaminating the marketed sprouts with electron beam or with other methods might be worth to prevent en masse infections.

Plant sprouts use up the protein, carbohydrate and fat stored in their seeds for their growth, until they are capable to photosynthesis. The nutritional values and mineral content of five plants (alfalfa, radish, wheat, mung bean and fenugreek) was identified and compared in their seeds and during germination (at the age of three and seven days) in reference to 100 grams.

The nutritional values and mineral content of seeds were usually multiples of the nutritional values and mineral content of the sprouts. During germination the protein, carbohydrate and fat content decreased. Radish sprouts had the highest vitamin C content (35.46 mg) which did not significantly change during germination. Of the minerals the iron content was higher in the seven day old sprouts, which makes it likely, that the iron stored in seeds only gets built in the tissues of the germinating plant later. The calcium content only increased in the wheat sprouts, it decreased or did not change in other sprouts during germination. This is probably because these minerals are built into the tissues it various times. The sprouts are usually consumed when they are seven days old, their nutritional content is therefore less, but their iron content is more, than the three days old sprouts. If they are not consumed on that day, but put into the refrigerator, storage on 4-6 ^{o}C for three days does not influence their nutritional values significantly. Comparing the nutritional values determined by us in the examined sprouts (alfalfa, radish, mung bean) with the American data reveals slight deviation. For lack of data only the nutritional values of three sprouts can be compared. Of the mineral contents the calcium and sodium content varied the most, the former was present at half the amount, the sodium content was present at multiple amounts in the sprouts examined by us compared to the American data, for which we found no explanation. Neither does the Hungarian table of nutrients contain information for the "raw", edible sprouts. For this reason our research might partially contribute to the expansion of the table.

When comparing the results of the spectrophotometric and the thin layer chromatographic examinations we can establish that the results of the two methods do not always check up, but might complement each other. The results of the spectrophotometric examinations suggest that C-glycosides are present in the highest amounts in the broccoli sprouts, but none of them were detectable on thin layer chromatograms. Similarly, in the case of mung bean sprouts, only the spectrophotometric method aimed at detecting C-glycosides indicated the presence of low amounts of flavonoids, but the thin layer chromatography did not confirm this finding, as neither the presence of the C-glycosides vitexin and orientin, nor of other flavonoids was corroborated. This is not surprising in view of those studies that reported flavonoids greatly contributed to the antioxidant capacity of legumes, for example vitexin and isovitexin in the case of the mung bean, however these accumulate not in the sprout but in the testa.

Our research tried to confirm, that valuable flavonoids in significant quantities can be present in sprouts, however we failed to substantiate this claim. Based on these research results we assume that the sprouts' antioxidant, antibacterial and other physiological effects are

primarily unattributable to the flavonoids. Based on the above we can infer that sprouts are an excellent source of ingested antioxidant phenolic compounds, but within the compound group the flavonoid group cannot contribute significantly to the antioxidant capacity. We also learnt that the active ingredient content and makeup of the various sprouts differs, and they can be included into a preventive diet with varying efficacy.

In our country the population's sprout consumption was not surveyed so far. Of the 200 people in our sample only 36 consumes sprouts, despite the fact that they can be grown at home or bought. The people in our sample consume sprouts monthly, with variable regularity, or even more infrequently, on occasion, primarily radish, alfalfa, corn and onion sprouts. Most of the pollee chooses from the sprouts based principally on their flavour. Their choice is significantly influenced by the physiological effects of the sprouts, and the nature of the food prepared from the sprouts. 80% of the responders would like to expand their knowledge regarding the sprouts, so they can form a full picture of their importance and applicability. In the everyday practice it has to be accentuated that plant sprouts can have a role in preventing diseases. As for their nutritional content they possess the same values characteristic of the vegetables used in everyday life, therefore their consumption is justified in a preventive diet, especially in the winter months. We aimed to contribute to the expansion of the population's knowledge, and the propagation of sprout consumption with the results of our research.

NEW FINDINGS

Our analysis presented in the thesis contains new findings and the possibility of their practical application, namely:

The effect of plant sprouts on bacteria

The antibacterial effects on enteric pathogens and other bacterial agents of fifty-five plant sprouts were analysed with microbiological methods. We have demonstrated that the radishes belonging to the Brassicacae family, kohlrabi and red cabbage sprouts inhibit the spread of the analysed bacteria strains, except for the *Pseudomonas aeruginosa*. 52% of the examined sprouts showed no antibacterial effect on any of the bacteria. The antibacterial homogenized sprouts in tenfold dilution do not inhibit the spread of bacteria. The decontamination of the seeds and sprouts is necessary when grown for and sold on the market.

Determination of the nutritional values of the sprouts

The nutritional values, together with the mineral content, of five sprouts were determined, of which three could be compared to the American data. With the determination of the nutritional values and mineral content of the sprouts we aimed to make up for the incompleteness of the Hungarian table of nutrients.

Survey of the consumption of dietary sprouts

The first Hungarian survey of the knowledge and consumption of sprouts was executed.

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